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Antimicrobial Susceptibility of Commercial Probiotic *Lactobacillus* Strains

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The intestinal microbiota plays an important role in normal gut function and maintaining host health. *Lactobacillus* sp. strains are lactic acid bacteria (LAB) and ubiquitous commensals of the normal human gut microbiota, and are widely explored as probiotics[1]. They express adhesiveness properties that enable them to inhibit the adhesion of bacterial pathogens to host cells, can produce biosurfactants, bacteriocins and other natural antibiotic molecules, and enhance immunological mechanisms against pathogens. Commercial strains - isolated or in blends - are available as nutritional supplements or even as medication, and both can be purchased without need of medical prescription in several countries[1,2].

A common application of probiotic lactobacilli strains is the administration to patients that are undertaking antimicrobial drugs in order to overcome side effects such as vomiting, and to provide some recovering of the normal microbiota at the disrupted ecological environment of the digestive tract[3].

Nevertheless, the behavior of *Lactobacillus* strains under the effects of antimicrobial drugs is poorly investigated, and it is technically difficult to predict the results of the concomitant use of lactobacilli and antimicrobial drugs[2-5]. Thus, here we investigate the susceptibility of commercial *Lactobacillus* strains to antimicrobial drugs widely used in hospital and homecare treatments of infectious diseases in Brazil.

Three comercial strains were used for this survey. Freeze dried aliquots of *L. paracasei* (SKL Pharma, Brazil) *L. rhamnosus* and *L. acidophilus* (Pharma Nostra, Brazil) were activated in MRS broth (Becton Dickinson, USA) in anaerobic jars for 48 h at 37 °C and then cultivated in MRS agar plates (Becton Dickinson, USA), in order to be used to prepare bacterial suspensions in McFarland 0.5 scale. As no standards exist for susceptibility testing of

lactobacilli, the following conditions were found to ensure confluent growth and thereby optimal susceptibility testing: a total of 100 μL of a suspension with density of McFarland 0.5 in 0.9% saline was spread on MRS agar plates (MRS agar was used in order to ensure good growth of the strains, as the standard Mueller Hinton agar failed to do so) and the following antimicrobial disks (all from Sensifar, Brazil) were used: Ampicillin (10 μg), meropenen (10 μg), gentamycin (10 μg), chloramphenicol (30 μg), levofloxacin (5 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), erythromycin (15 μg), nitrofurantoin (300 μg), and sulfamethoxazole-trimethoprim (25 μg). The plates were then incubated in anaerobic jars (microaerophilic environment) for 24 h at 37 °C, and inhibition zones were measured.

Results are summarized in table 1. Our study shows different features of others that demonstrated resistance of LAB to most of the drugs we tested. It is desirable that probiotic bacteria resist to exposure to antimicrobial drugs, such that they can be co-administered.

L. rhamnosus strain was the only one that was susceptible to all tested drugs and large inhibition zones were detected; therefore, it is possible that the administration of this strain and the tested antimicrobials will result in poor probiotic effects expected after administrating the LAB. Nevertheless, *in vivo* experiments are needed for confirmation of this hypothesis.

Interestingly, *L. acidophilus* and *L. paracasei* strains were not susceptible to quinolones, what suggest that they might be useful for patients in pharmacological treatment of urinary tract infections and other conditions such as sinusitis, in which quinolones are first-choice drugs.

Table 1 – Antimicrobial susceptibility results

Antimicrobial Drug	***L. rhamnosus***	***L. acidophillus***	***L. paracasei***
GEN	35	0	0
MER	50	29	40
AMP	35	22	26
SUT	40	0	0
LEV	42	0	0
CHLO	32	26	26
ERI	35	23	30
NIT	23	26	22
CIP	42	0	0
NOR	42	0	0

GEN: Gentamicin; MER: Meropenem; AMP: Ampicillin; NIT: Nitrofurantoin; SUT: Sulphamethoxazole-trimethropim; LEV: Levofloxacin; CHLO: Chloramphenicol; ERI: Erithromycin; CIP: Ciprofloxacin; NOR: Norfloxacin. Average measurements of inhibition zones are expressed in mm. Experiments were performed in duplicate.

Also, *L. acidophilus* and *L. paracasei* strains were not susceptible to gentamicin and to Sulphamethoxazole-trimethropim. Gentamicin is a broad spectrum aminoglycoside that work by binding to the bacterial 30S ribosomal subunit, causing misreading of t-RNA, imparing protein synthesis. As aminoglycosides are generally ineffective against anaerobic bacteria, this resistance was somehow expected. Sulfamethoxazole is a bacteriostatic drug that inhibits bacterial synthesis of dihydrofolic acid by competing with para-aminobenzoic acid for binding to the enzyme dihydrofolate synthetase. Trimethoprim is a dihydrofolate reductase inhibitor, which impairs the reduction of dihydrofolic acid to tetrahydrofolic acid. Bacterial resistance tend to be slower when these drugs are used in combination[6,7].

The alteration of the intricate networks of the normal gut microbiota by antimicrobial use is a well recognized condition, and ingested probiotics can help the organism to reestablish the normal microbiota[3]. Antimicrobial susceptibility testing of lactobacilli is important for checking the biosafety of potential probiotic strains for clinical use, beyond, for instance, the detection of pathogenic or virulence properties[1,2,7]. The spread of antimicrobial resistance determinants from bacteria used in probiotic products is a real risk, although intrinsic drug resistance of lactobacilli have been described to be not associated to mobile genetic elements, but to chromosomal genes. It has been described, nevertheless, that susceptibility to antimicrobial drugs is often species-dependent among *Lactobacillus* gender[5-7].

Certain limitations can be mentioned to antimicrobial susceptibility of LAB. MRS agar has been used in similar researches worldwide, but little is still known about the interaction of MRS and antimicrobial drugs. There is still a lack of agreement on the interpretative breakpoints for probiotic bacteria, and antimicrobial susceptibility data of *Lactobacillus* strains are scarce. Suitable criteria for susceptibility tests are not available until the preparation of this manuscript, what makes it difficult to define drug resistance patterns[6].

Broth microdilution has been explored as an alternative to overcome this difficulty, but non-parameterized MICs and breakpoints are still a technical problem[4-7]. Given these situations, the scientific community expects that the CLSI, EUCAST and other expert committees on antimicrobial testing provide soon the needed parameters for antimicrobial susceptibility of these bacteria, given their clinical relevance as probiotics.

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